

# CALCULATION EXAMPLES FOR BRADFORD AND ELISA

## **BRADFORD PROTEIN ASSAY**

- (1) After running the assay, you will have a value that gives you a protein concentration in a well of between 0-5 ug.
- (2) You take this number and divide it by the number of ul of sample that you placed in the well, giving you a ug/ul value.
- (3) You then multiply this number by the number of ul that you extracted (figured out by measuring the amount of extract you have using a graduated cylinder), resulting in an ug extracted value.
- (4) Finally, you divide this number by g weight that you extracted and you now have a ug/g value that can be converted in a mg/g value by dividing by 1000

Example:

OD reading = 0.306

ug/well concentration = 2.54 ug/well

ul of sample/well = 10 ul/well

amount of extract = 7650 ul

weight extracted = 1.0 g

$$2.54 \text{ ug/well} \div 10 \text{ ul/well} \times 7650 \text{ ul} \div 1.0 \text{ g} = 1943.1 \text{ ug/g} = 1.9431 \text{ mg/g}$$

## **ELISA**

### **Calculation used to determine amount of sample needed for each well**

- (1) Take the ug/ul value obtained after step 2 from the Bradford protein assay and divide it into 0.02 ug (which is a value in the middle of the ELISA curve). This gives you the number of ul needed to give a concentration of 0.02 ug/well. By targeting a concentration of 0.02 ug/well, you can dilute a little more or a little less per sample so you can standardize your dilutions. In other words, the number of ul calculated could be 2.16, 3.04, 2.78 for three different samples and you could just use 2.5 ul for each.
- (2) Place this amount of sample in a well and add enough PBS to equal a total volume of 50 ul or pre-dilute the sample by multiplying the amount needed per well by 20 (since there are twenty 50 ul amounts in 1 ml). Add this to a microfuge tube with enough PBS to equal 1 ml (1000 ul).

Example: (from above)       $2.54 \text{ ug/well} \div 10 \text{ ul/well} = 0.254 \text{ ug/ul}$   
                                     $0.02 \text{ ug} \div 0.254 \text{ ug/ul} = 0.079 \text{ ul}$   
                                    (Value of 0.1ul might be a logical amount for this result.)

## PROTEIN CONCENTRATION CALCULATION FROM **ELISA** VALUE

- (1) After running the assay, you will have a value that gives you a protein concentration in a well of between 0-0.04 ug.
- (2) You take this number and divide it by the number of ul of sample that you placed in the well, giving you a ug/ul value.
- (3) You then multiply this number by the number of ul that you extracted (figured out by measuring the amount of extract you have using a graduated cylinder), resulting in an ug extracted value.
- (4) Finally, you divide this number by g weight that you extracted and you now have a ug/g value that can be converted in a mg/g value by dividing by 1000

Example: (continued from above)

OD reading = 0.759

ug/well concentration = 0.016 ug/well

ul of sample/well = 0.1 ul/well

amount of extract = 7650 ul

weight extracted = 1.0 g

$$0.016 \text{ ug/well} \div 0.1 \text{ ul/well} \times 7650 \text{ ul} \div 1.0 \text{ g} = 1224.0 \text{ ug/g} = 1.224 \text{ mg/g}$$

By comparing this value with the total protein value obtained from the Bradford assay, the amount of the protein that is immunoreactive can be determined, giving a % IR (immuno-reactive).

Example: (continued from above)

$$\begin{aligned} \% \text{ IR} &= (\text{immuno-reactive protein concentration} \div \text{total protein concentration}) \times 100 \\ &= (1.224 \text{ mg/g} \div 1.9431 \text{ mg/g}) \times 100 = 62.99 \% \end{aligned}$$